

## MORPHOTOXICITY OF FUNGICIDE MANCOZEB ON TWO GENOTYPES OF VIGNA

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### ABSTRACT

Fungicides are expected to protect crop plants from fungi infection without harming the crop plants. Mancozeb (MZ), [[1,2-ethanedithiolbis-[carbamodithio-ato]] (2-)] manganese, mixture with [[1,2-ethanedithiolbis-[carbamodithioato]] (2-)] zinc, is a fungicide of the carbamate pesticide family. Registered for use on a variety of vegetables, fruits, nuts and grain crops, it is also used for seed treatment of cotton, potatoes, corn, safflower, sorghum, peanuts, tomatoes, flax, and cereal grains. In present study, toxic influence of mancozeb, commonly used fungicide in agricultural practices was checked on seed and seedling growth of two pulses namely *Vigna radiata* and *V. mungo*. The objective of this study is to determine the rate of germination, survival, root shoot ratio, tolerance index, vigour index and phytotoxicity of the two genotypes seeds exposed to eight concentration of MZ viz. 10, 30, 50, 70, 90, 110, 130 and 150 ppm assessed after 7 and 15 days of fungicide treatment. In general the seeds of both *Vigna* genotypes showed a marginal decrease in the rate of germination, survival, root shoot ratio, tolerance index, vigour index and phytotoxicity in 10 ppm over the control. There was gradual and significant decrease in rate of germination, survival, root shoot ratio, tolerance index, vigour index and phytotoxicity at higher concentration concentrations of mancozeb. The maximum inhibition was observed in higher concentration of mancozeb (150 ppm). But the results showed that increasing mancozeb concentrations caused reduction in all the studied parameters of seedlings in both the genotypes but response was different among genotypes.

**KEYWORDS:** Fungicide, Mancozeb, Phytotoxicity, Seed Treatment, *Vigna radiata*, *Vigna mungo*, Tolerance Index & Vigour Index

**Received:** Jan 08, 2017; **Accepted:** Feb 15, 2017; **Published:** Feb 17, 2017; **Paper Id.:** IJBRAPR20172

### INTRODUCTION

Pulses, also known as grain legumes, belong to family Fabaceae and are the most important food crop of very ancient origin associated with human existence, survival and their socio-economic history. Farmed legumes can belong to many agricultural classes, including forage, grain, blooms, pharmaceutical/industrial, fallow/green manure and timber species. Grain legumes are cultivated for their seeds. The seeds are used for human and animal consumption or for the production of oils for industrial uses as are a significant source of protein, dietary fiber, carbohydrates and dietary minerals.

Black gram (*Vigna mungo* L.) and green gram (*Vigna radiata* L.) are two important pulse crops of India since ancient time as both the crops play a very important role in human diet as a rich source of protein. India is one of the principal pulse growing countries of the world with an area of about 23.9 million hectares and with an average production of 15.8 million metric tons/year. Despite growing pulses in large area, the production and

average yield/hectare is quite low, mainly due to the losses carved by pests and diseases (Chand et al., 2016). There are a number of approaches for disease management, such as changes in cultural practices (Coker et al., 2001), use of resistant cultivars (Bates et al., 2008; Dorrance et al., 2003), and the use of fungicides (Coker et al., 2010; Munkvold 2009). Seed treatment with fungicide has been recommended while planting during conditions conducive for the development of seedling diseases or planting under conservation tillage (Poag et al., 2005; Popp et al., 2010) and also for storage. In pulses, the efficacy of fungicide seed treatment has been demonstrated, showing increase in stands and yields (Bradley 2008). Munkvold (2009) reported that the use of fungicide seed treatments in soybean increased from 8% in 1996 to 30% in 2008. Thus the concept of fungicide seed treatments to reduce the incidence of seed-borne diseases in crops and also to protect seeds from infection by soil-borne fungi, therefore leading to establishment of healthy and vigorous plants resulting in increased yields (Urrea et al., 2013).

Mancozeb (MZ), [[1,2-ethanediybis-[carbomodithio-ato]] (2-)] manganese, mixture with [[1,2-ethanediybis-[carbomodithioato]]-(2-)] zinc, is a fungicide of the carbamate pesticide family. It is an organic contact fungicide having preventive action by killing or inhibiting fungi or spores before the mycelia grow and develop within the plant tissues (Yuste and Gostinear, 1999). Registered for use on a variety of vegetables, fruits, nuts and grain crops, it is also used for seed treatment of cotton, potatoes, corn, safflower, sorghum, peanuts, tomatoes, flax, and cereal grains (Dhanamanjuri, 2013). Thus it is fairly effective against external contamination with seed-borne smut fungi and surface borne *Helminthosporium* stripe of barley. They undergo transformation to ethylene diisothiocyanate, which inactivates thiol groups of enzymes and metabolites in fungal cells. These compounds control broad range of fungi at relatively low application rates (Dias, 2012). Its morphotoxic and phytotoxic effect has been reported by Anitha and Savitha (2013) in rice seedlings. Mancozeb was found to reduce populations of *Plasmodiophorabraceae*, the cause of club root in crucifers (Sinha et al., 1988). In chilli mancozeb application showed adverse effect in the form of drying, necrosis and shredding of leaves and wilting of plants (Kapgate et al., 2008). The effect of fungicide, mancozeb was assessed based on germination and seedling morphological traits; root and shoot length (Sengupta et al., 1986). Literature is rich in information referring to the phytotoxic and inhibitory effects of fungicide on germination root and shoot growth (Pablo, 2003; Sathees et al., 2014). The effects of fungicide on seed germination, growth, survival, and yield and residue quantification of crop plants have been investigated by Ahmed and Khan (2011).

In present study *Vigna mungo* L. var. T9 and *Vigna radiata* var. PDM139 were used for testing the toxicity of fungicide. As fungicides are considered useful to eliminate pests, but can also induce negative effects to the processes of germination, growth and development of plants or disrupt some physiological and metabolic processes. Thus by studying the effect of fungicide on treated seeds for germination and determination of growth indices, it is possible to evaluate the positive or negative influence or toxicity of the chemical compounds. Because germination experiments are easy and less time consuming for testing the action of some known and newly synthesized substances on living organisms. Since an understanding of hazardous effect of pesticides is also as equally as important to that of their use in the plant protection.

## MATERIALS AND METHODS

### Chemicals

Mancozeb, MZ (75% W.P) manufactured by Hindustan Agrisciences, was purchased from a local agricultural store in Lucknow, India. The other chemicals used in the present study were of analytical grade.

### Plant Material and Pesticide Treatment

The seeds of two *Vigna* species, *Vigna mungo* L. var. T9 and *Vigna radiata* var. PDM139 were purchased from Gomti Pharma, Lucknow, India. Healthy and equal size seeds were selected and surface sterilized by thoroughly washing with distilled water. Seeds were incubated for 24 h in different concentrations of MZ viz. 10, 30, 50, 70, 90, 110, 130 and 150 ppm and distilled water which served as control. After 24 h the control and treated seeds were placed on the double layers filter paper wet with distilled water in petridish. These were incubated at  $25\pm 1^{\circ}\text{C}$  for 15 days with regular supply of distilled water. Three replicates for each concentration were maintained along the control for comparison and each replicate had 20 seeds per petridish.

### Morphological Analysis

Different parameters of *Vigna* seedlings were investigated like seed germination percentage (G%), survival percentage (S%), root shoot length ratio (RSL ratio), seedling vigor index (SVI), percentage of phytotoxicity (P%) and tolerance index (TI). The germination percentage was taken on 3<sup>rd</sup> day after sowing on filter paper by counting the number of seeds germinated out of total number of seeds treated (Scott et al., 1984; Akinci and Akinci 2010). After 7 days of germination, S% was calculated as the ratio between the total number of seedlings survived and total number of seed treated. On 15<sup>th</sup> day, the root and shoot length was measured and their ratio was also calculated. The SVI is a property of the seed, assaying the level of activity of the seed during germination and seedling emergence. It was calculated on 15<sup>th</sup> day as described by Abdul Baki and Anderson (1973). The percentage of phytotoxicity and TI was calculated on 15<sup>th</sup> day according to Chou et al., (1978) and Turner and Marshal (1972) respectively.

### Statistical Analysis

All the data was analyzed by SPSS version 15 statistical software for windows for one-way analysis of variance (ANOVA) followed by a Duncan's multiple range test (DMRT).

## RESULTS

The results of the present study presented in Figure 1, depicts differential responses of two *Vigna* genotypes which were treated for 24 h with various MZ concentrations. Generally, a significant reduction in germination, survival, root and shoot growth parameters was observed at higher concentrations of MZ than lower concentrations and control treatments.

The mean value for germination percentage (G %) of the two *Vigna* genotypes differed significantly after fungicide stress induced by MZ treatment. Treatment with MZ led to a significant dose dependent decrease in germination percentage in both the genotypes. Maximum G% (100%) was recorded in control for both genotypes which reduced to 95% and 88.33% for *V. mungo* and *V. radiata* respectively for minimum concentration of MZ (10 ppm). MZ caused maximum percent decrease over control in G% at 150 ppm of *V. mungo* and *V. radiata* where germination was reduced up to 33.33% and 28.33% respectively. The MZ concentration which led to about 50% inhibition over control in germination was different for the two genotypes. It was between 130-110 ppm for *V. mungo* and was between 110-90 ppm for *V. radiata*.

The seedling survival percentage (S %) showed a marked significant dose dependent decrease in the two *Vigna* genotypes. The S% of seedlings at the maximum MZ concentration (150 ppm) was 31.66 in *V. mungo* and 28.33 in *V.*

*radiata*. In *V. mungo*, maximum S% was in control (100%) and minimum at 150 ppm (31.66%) with percent reduction of 68.34%. In *V. radiata* maximum S% was in control (96.66%) and minimum at 150 ppm (28.33%) with percent reduction of 71.67%.

The effect of different concentrations of MZ on shoot and root length was found to be statistically significant and there was dose depended decrease with treatment at various concentrations of MZ for both the genotypes. The RSL ratio, being important for functional balance between photosynthesis and water absorption by the roots, showed a significant dose dependent decrease for MZ treatment. The RSL ratio was highest in control for *V. mungo* (1.58) and *V. radiata* (1.74) which gradually decreased to 1.09 and 1.02 for *V. mungo* and *V. radiata* respectively. Seedling vigour index (SVI) decreased significantly with increasing concentration of fungicide MZ in all the treatments and genotypes. The maximum SVI 2703.33 and 2340 was observed in control of *V. mungo* and *V. radiata* respectively on 15<sup>th</sup> day of treatment. The minimum SVI 423.83 and 267.66 was observed at 150 ppm concentration of MZ in *V. mungo* and *V. radiata* respectively.

The maximum tolerance index (TI) was observed in *V. mungo* (0.93) and *V. radiata* (0.94) at 10 ppm concentration of MZ. TI was found to decrease significantly and dose dependently, with minimum TI being observed in 150 ppm. The minimum TI calculated for *V. mungo* was 0.36 and for *V. radiata* it was 0.4.

The percentage of phytotoxicity (P%) calculated on 15<sup>th</sup> day of treatment was minimum at 10 ppm for both the genotypes which further increased significantly and dose dependently. For *V. mungo* and *V. radiata* maximum P% was 1003.93% and 709.55% respectively, while minimum percentage of phytotoxicity was observed at 10 ppm for *V. mungo* and *V. radiata*.

## DISCUSSIONS

Fungicides represent one of the most effective and integrative methods of controlling diseases by acting against phytopathogenic fungus in agriculture. However, the toxicity and the pollution generated by fungicides cannot be neglected, and their toxic effect on seeds depends on their distribution, concentration, persistence and metabolism of its active ingredients (Petit et al., 2012). To analyze the stress created by fungicide MZ, petridish bioassay was used for germination test that allows simple and inexpensive analysis of biological activity of environmental pollutants, like fungicides and other hazardous agrochemicals (Beckie et al., 1990; Ksahani et al., 2007; Perez and Kogan, 2003). In this study effect of MZ on seed germination was assessed in two genotypes of *Vigna* and it was shown that even lowest concentration of MZ used was inhibitory for seed germination and root growth while at higher concentrations stunting of roots and reduction of growth was observed.

According to our results various concentrations of MZ inhibited the G% of *Vigna* seeds by varying degrees. This may be attributed to the adverse effect of the MZ on degradation and mobilization of seed reserves (Amar and Reinhol, 1973). Earlier studies suggested that toxicant produced by fungicide application may retard the protein and carbohydrate synthesis by inducing alteration in cytochrome oxidase activity, blocking alternative respiratory pathways (Siddiqui and Ahmed, 2002). The S% calculated on 7<sup>th</sup> day was found to decrease significantly and dose dependently, as also reported in soya bean and mung bean genotypes due to the stress of pesticides (Basantani et al., 2011; Marinov, 2009). However S% was higher in *V. mungo* as compared to the *V. radiata* at maximum (150ppm) MZ concentration, thus indicating that *V. mungo* is more tolerant than *V. radiata*.

The effect of MZ on root and shoot length was found to be statistically significant at various concentration of MZ. In *Vigna* genotypes, as compared to control, the root and shoot length was decreased with increased concentration of fungicide. As a result the RSL ratio also decreased significantly and dose dependently. Minimum RSL ratio was recorded at maximum concentration of MZ. According to Ahmad and Khan (2011) plant growth is affected by an osmotic shock effect due to fungicide which cause release in structural protein and loss of transportability in the leaf cells.

SVI was calculated by determining the germination percentage and seedling length of the seeds which was found to decrease significantly and dose dependently. Similar result was reported by Ashagre (2013) in their experiment on seed germination and seedling growth of haricot bean cultivars showing phytotoxicity of shoot and root length at higher concentration of copper sulphate. According to Abdul-Baki and Anderson (1973) the seed lot showing the higher SVI is considered to be more vigorous. While comparing the SVI of both the genotype of *Vigna*, *V. mungo* was found to be better performer in stress than *V. radiata*. The TI calculated on 15<sup>th</sup> day also decreased significantly and dose dependently. Abiotic stress causing decline in SVI and TI has been reported in many crops such as *Cicerarietinum* (Narain, 2012), spinach (Bijeh et al., 2011) and wheat (Datta et al., 2009; Hussain et al., 2013).

The P% calculated on 15<sup>th</sup> day gives an overview on the toxicity of fungicide on the *Vigna* genotypes which could be expressed as an injurious effect of fungicide. Similarly, many fungicides showed phytotoxicity to different field crops under various conditions of their application (Ali and Archer 2003; Singh et al., 2003). In present study the P% was found to increase dose dependently and significantly for both genotypes, but while comparing the two genotypes, *V. mungo* was more affected by the fungicide MZ than *V. radiata*.

## CONCLUSIONS

Thus it can be concluded that at highest dose, germination percentage was greatest for *V. mungo* and minimum for *V. radiata* and similar result was found for survival percentage. Minimum vigour index at 150 ppm was in *V. radiata* and maximum in *V. mungo*. Thus by studying the effect of fungicide on treated seeds germination and determination of growth indices, it was possible to evaluate the toxicity of MZ. If both genotypes compared on the basis of performance at higher doses for all the parameters it can be concluded that *V. mungo* is better performing under fungicide stress as compared to *V. radiata*. It is quite obvious that fungicides remain an essential tool for plant disease management and will continue to play a crucial role in optimizing yields from crops. Therefore a critical examination of the toxicity of different fungicides is necessary in order to optimize their doses and also to develop combinations which are effective even at very low concentrations which will provide benefits not only for plants yield but also for the environment and human health.

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## APPENDICES

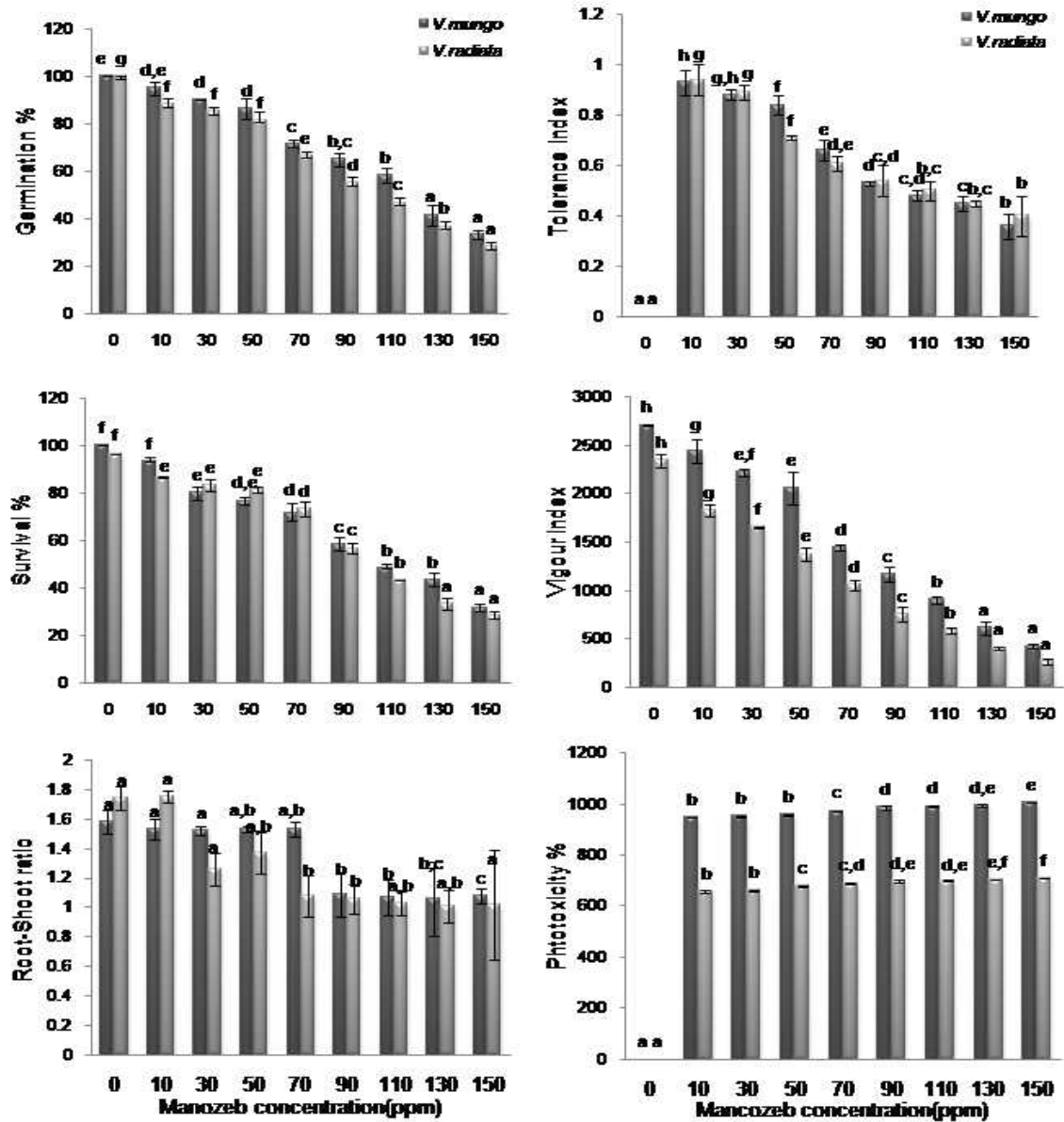


Figure 1: Effect of Mancozeb on the Morphological Parameters of *Vigna Mungo* and *Vigna Radiata* Seedlings. Data Represent Means  $\pm$  S.E. of 3 Replicates and Data with different Superscripts are Significantly Different at  $P \leq 0.05$ , as Determined by ANOVA Followed by Duncan's Multiple Range Test